Research Article

The effect of different concentrations of copper sulfate on the some physiological and immunological parameters of local male rabbits

Asif Hasan Abdulrazzaq[1], Saad Thabit Jassim Alrawi[2]*, Abdulwahab Bedawi Alkubaisi[3]

ABSTRACT

Background: Copper is an essential trace element and necessary for body survival. It plays a role in red blood cells construction. Additionally, it act as a cofactor in various critical biological manners such as iron transport and respiration. As well as, the other main functions of Cu are enzymes cofactor, antioxidant enzymes, and non-enzymatic antioxidants such as cytochrome oxidase, lysyl oxidase, ceruloplasmin, superoxide dismutase, catalase, glutathione, and metallothionein. **Materials and Methods:** This study was conducted to investigate the role of different concentrations of copper sulfate on the immunity response. A total of 24 local male rabbits were divided into three groups. The first and second groups were administrated copper sulfate orally 0.75% and 1%, respectively, while the third group was administrated normal saline 1% as a control group. **Results:** The results showed a highly significant effect ($P \leq 0.05$) for IgG, IgM, and IgA in 0.75% concentration of copper sulfate compared with T1 and T3 and so showed a significant increment of IgG and IgA concentration in the serum of rabbit Group 1 (T1) compared with control Group T3. On the other hand, the results showed a significant ($P \leq 0.05$) increase in the complement C3 of rabbit that administrated 0.75% and 1.0% of copper sulfate compared with control Group T3. Moreover, the results revered to a significant effect in complement concentration C4 in Group T2 compared with T3. The results recorded significantly decrement in the reduction of dye (NBT) in the two treated groups compared with the control group T3. While the migration inhibition factor, there were recorded no significant differences among the treated groups compared with the control group. The liver and spleen weight of treated group rabbits were showed significant increases compared with the control group. **Conclusion:** The study concluded that the copper sulfate has effectiveness role an immunity improvement.

KEY WORDS: Copper sulfate, Immunity, Migration inhibition factor, NBT, Rabbit

INTRODUCTION

Copper is an essential trace element and necessary for body survival. It plays a role in red blood cells construction.[1] Likewise, helps as a cofactor in different basic natural habits, for example, iron transport and respiration.[2] As well as, the other main functions of Cu are enzymes cofactor, antioxidant enzymes, and non-enzymatic antioxidants such as cytochrome oxidase, lysyl oxidase, ceruloplasmin, super oxidase dismutase, catalase, glutathione, and metallothionein.[3,4] Copper contributed with many important interactions of the body and also has a strong relationship in stimulating the immune system.[5] It might induce an innate immune response is by direct stimulation of toll-like receptors (TLRs).[6] While others research shows that the copper can induce a higher concentration and regulation of the immune markers such as (complement, neutrophils, and macrophages of both M1- and M2-phenotypes)[7] and true conversely. Copper deficiency can cause, anemia and growth combat and loss of some metabolic enzymes such as superoxide dismutase. Immune response inhibition, neutropenia, decrease of white blood cells, or neutrophils and decreasing lymphocyte cells may also resulted from copper deficiency.[8] On the other hand, increasing copper rates, it may lead to poisoning and the occurrence of chest pain, abdomen pain, stop urination, yellowish skin, may be developed severe intravascular hemolysis, acute severe hepatic and renal failure, as well as adrenal insufficiency.[9] Therefore, this study conducted to

Access this article online	
Website: jprsolutions.info	ISSN: 0975-7619

[1]Department of Biotechnology, College of Applied Science, University of Fallujah, Iraq, [2]Department of Public Health, College of Veterinary Medicine, University of Fallujah, Iraq, [3]Department of Pathological Analysis, College of Applied Science, University of Fallujah, Iraq

***Corresponding author:** Saad Thabit Jassim Alrawi, College of Veterinary Medicine, University of Fallujah, Iraq. E-mail: saadalrawe50@gmail.com

Received on: 11-06-2019; Revised on: 17-07-2019; Accepted on: 22-08-2019

evaluate the role of copper in the immune response and improvement.

MATERIALS AND METHODS

A 24 local male rabbit weights 950–1200 g were divided randomly into three groups; each group has eight rabbits. The first and second groups were administrated copper sulfate orally 0.75% and 1%, respectively, while the third group was administrated normal saline 1% as a control group. All animals were fed ration *ad libitum*. Blood samples were collected from the heart after 30 days and the following parameters were measured:

Concentration Rate of Immunoglobulins and Complement System Proteins

Single radial immunodiffusion were used to measure the concentration of immunoglobulines and complement using according to manufacturer instructions (LTA/Italy).[10]

Nitroblue Tetrazolium Reduction Test (NBT)

Add 75 µl from polymorphonuclear leukocytes (PMNs) suspending in special glass tubes then add 75 µl of NBT dye. Mix the contents quietly between the fingers and put the tubes in the incubator at 37°C for 25 min, then take 20 µl from each tube and put on a slide, left to dry and fixed with methanol solution and then dye by Giemsa stain leave 15 min. Then, cells calculated under the optical microscope.[11]

Migration Inhibition Factor (MIF) Test

Pour 10 mL of the agarose medium in the Petri dish and then left to harden, then done two holes for each Petri dish. After that, 200 µl of PMNs concentration of 1 × 10 cells/mL were placed in each hole, incubation 37°C with 5–10% CO_2 for 32 h. After the incubation, fixed them with methanol solution for 15 min, dyed by Giemsa stain for 20 min and then rinse with distilled water, calculate MIF by Ruler.[12]

Statistical Analysis

The data obtained were subjected to statistical analysis using one-way analysis of variance and least significant differences *post hoc* test was performed using SPSS-24.

RESULTS AND DISCUSSION

The results showed that the different copper sulfate concentrations had has effects on the immune response; furthermore, stimulation humoral and cell-mediated immune system were observed, copper sulfate had an effect on the immunoglobulin and complement concentrations in treated rabbits serum and showed a highly significant ($P \leq 0.05$) concentration (IgG) for all groups treated with copper sulfate compared with the control group T3 [Table 1]; furthermore, the results showed a highly significant ($P \leq 0.05$) concentration of IgM and IgA in T2 compared with T1 and T3 and showed a highly significant ($P \leq 0.05$) (IgA) concentration in T1 compared with control group T3. Also the results revealed that there is a significant effect in 0.75% concentration of copper sulfate, these result agreements with[13,14] that indicate the optimum dietary copper sulfate levels could be greater than 10.3 mg Cu kg^{-1} diet but less than 13.1 mg Cu kg^{-1} diet in juvenile beluga, when copper sulfate is used as the dietary source of inorganic copper. also, agreement with Senthilkumar P *et al*.[5] that the levels of Ig, IgG, and IgM against chicken RBC have a tendency to be higher in copper sulfate administrated to lambs as role of copper in immunity response. This results can be explained through that important chemical element necessary for survival and great importance for this element by enters in the bioactivity of the organism.[15,16] furthermore, "copper has been known to have antimicrobial activity, it has been recognized by the American Environmental Protection Agency as the first metallic antimicrobial agent in 2008"[17] and used to stimulate the immune response by affecting on TLR receptors on the surfaces of immune defense cells that considered the first line of defense.[6] Moreover, the expression of interleukin-1-beta (IL-1b) and tumor necrosis factor-alpha (TNF) showed a dose-dependent increase with the increased copper sulfate dose.[18] As well as, the results recorded a highly significant ($P \leq 0.05$) in the complement C3 of the Group T1 and T2 compared with T3, and increase significantly in the complement C4 in the Group T2 compared with T1 and T3, also found a highly significant in Group T1 than T3 [Table 1]. That consideration is one of the most important factors in innate immunity, the functioning of the complement system during the early stage of infection and directly mediates the elimination of pathogens.[19] The results agreed with El Basuini *et al*.,[16] which shows a significant effects ($P \leq 0.05$) of copper-loaded nanoparticle supplementation on growth performance, hematological and immunological properties in broiler herbs, especially for complement C3 and C4.

The results showed a decrease significantly ($P \leq 0.05$) in the reduction of dye (NBT) in the two treatment groups with copper sulfate compared with the control group T3 [Table 2]. NBT is an interesting material that is of importance in the study of neutrophil capacity and valuable to the study of phagocytosis. The results of the current study disagreement with the Sharma *et al*.,[20] who mentioned that the neutrophils have highly phagocytic activity against *Candida albicans*, explain this result here is the presence of the causative agent or foreign body (bacteria, fungi, and virus) and the animal treatment with copper sulfate. However, our results agree with Zvyagintseva *et al*.[21]

Table 1: The effect of copper sulfate on some component of immunity on the serum rabbits

Treated group	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)	C3 (mg/dl)	C4 (mg/dl)
T1 (1.0) $CuSO_4$	1946.67±17.64[a]	181.0±9.24[b]	322.5±18.18[b]	359.0±15.01[a]	74.0±2.31[b]
T2 (0.75) $CuSO_4$	2203.0±32.47[a]	230.0±20.78[a]	532.5±22.27[a]	358.0±5.77[a]	92.0±2.88[a]
T3 control	933.33±60.09[b]	182.0±4.16[b]	202.83±41.57[c]	214.66±44.78[b]	46.0±6.92[c]

The different lower case letters refer to significant variances between different groups at $P \leq 0.05$

Table 2: The effect of copper sulfate on the spleen, liver weight, MIF, and NBT on the serum rabbits

Treated group	Spleen weight (g)	Liver weight (g)	MIF (mm)	NBT (%)
T1 (1.0) $CuSO_4$	0.77±0.082[a]	51.31±136[a,b]	17.17±1.61	7.66±1.66[c]
T2 (0.75) $CuSO_4$	0.29±0.13[b]	64.29±8.46[a]	17.12±1.73	12.66±1.45[b]
T3 control	0.53±0.02[a,b]	40.32±6.48[b]	15.61±0.98	16.34±1.20[a]

The different lower case letters refer to significant differences between different groups at $P \leq 0.05$

during the copper administration of investigational animals showed a decline in the guide of completion of phagocytosis, indicating the letdown of the endocytosis process and low stimulation index due to the low activity of the NADPH oxidase system of phagocytes. Moreover, copper sulfate, especially in low concentration, is an important factor in the immune system.

The results of the MIF recorded a non-significant effect among the treated compared with the control group. The results of the Migration Inhibition factor (MIF), recorded a non-significant effect among the treated compared with the control group. Perhaps the interpretation of this result that copper sulfate is an immune stimulation or enhancement and enter in oxidative processes also a metabolic stimulant and enter into the synthesis of many important enzymes in metabolic activity[16]. copper sulfate increases the activity of macrophage cells[22], and this leads to an increase in expression and production IL-1beta, TNF-alpha[13], they are important sources to inhibition polymorphic cells (PMNs) to migration from capillary.[23] The macrophage cells that consider a source of MIF, so usually the mice that appear as a result of MIF deficiencies are protected from the consequences of infection.[24,25]

It was also found that the significant effect of results had a significant ($P \leq 0.05$) effect on the liver weight of rabbits that treated with copper sulfate T2 compared with treatment T3. May be due to the copper sulfate has effectiveness showed off in all biological activities and also the copper useful in improved final body weight in broiler chicken.[26] Liver is an important organ affected by trace chemical elements, as it has an effect on the metabolic effectiveness, including in the metabolism of all kinds of lipid profile. Samanta *et al*.[27] displayed that, there is significant reductions ($P \leq 0.05$) of total cholesterol, triglyceride, and an elevated concentration of HDL-cholesterol, of the chickens fed with 250 mg Cu kg^{-1}. Copper deficiency has been reported to influence lipid metabolism"[28] as well as has to effect on liver enzymes such as cholinesterase (ChE) that found to be affected by copper sulfate, as 100% of cholinesterase activity was inhibited at 20.0 mg/L.[29]

The spleen weight of rabbits that treated with copper sulfate Group T1 recorded significantly increased compared with the control group T3. Spleen considered as an important immune organ by active immune response through humoral and cell-mediated pathways. These results agreement with Mitra *et al*.[30] who mention related decreases and increases, respectively, in splenic weights that depended on copper-treated mice proved immunotoxicity as indicated when used 5 mg/kg and splenic weight increased depended on rising copper concentration.

CONCLUSION

The results revealed that the using of copper sulfate leads to improve some immunity component.

REFERENCES

1. Johnson MA, Fischer JG, Kays SE. Is copper an antioxidant nutrient? Crit Rev Food Sci Nutr 1992;32:1-31.
2. Calafato S, Swain S, Hughes S, Kille P, Stürzenbaum SR. Knock down of *Caenorhabditis elegans* cutc-1 exacerbates the sensitivity toward high levels of copper. Toxicol Sci 2008;106:384-91.
3. Lim HS, Paik IK. Effects of dietary supplementation of copper chelates in the form of methionine, chitosan and yeast in laying hens. Asian-Australas J Anim Sci 2006;19:1174-8.
4. Gopi N, Vijayakumar S, Thaya R, Govindarajan M, Alharbi NS, Kadaikunnan S, *et al.* Chronic exposure of *Oreochromis niloticus* to sub-lethal copper concentrations: Effects on growth, antioxidant, non-enzymatic antioxidant, oxidative stress and non-specific immune responses. J Trace Elem Med Biol 2019;55:170-9.
5. Senthilkumar P, Nagalakshmi D, Ramana Reddy Y, Sudhakar K. Effect of different level and source of copper supplementation on immune response and copper dependent enzyme activity in lambs. Trop Anim Health Prod 2009;41:645-53.
6. Rachmawati D, Bontkes HJ, Verstege MI, Muris J, von Blomberg BM, Scheper RJ, *et al.* Transition metal sensing by toll-like receptor-4: Next to nickel, cobalt and palladium are potent human dendritic cell stimulators. Contact Dermatitis 2013;68:331-8.
7. Trindade R, Albrektsson T, Galli S, Prgomet Z, Tengvall P, Wennerberg A, *et al.* Bone immune response to materials,

part II: Copper and polyetheretherketone (PEEK) compared to titanium at 10 and 28 days in rabbit tibia. J Clin Med 2019;8:814.
8. Abd-Alrazaak AH. Study the effect of some immunological aspects on Newzealand rabbits drenched with different concentrations of copper sulfate and zinc. Kufa J Vet Med Sci 2011;2:47-53.
9. Sinkovic A, Strdin A, Svensek F. Severe acute copper sulphate poisoning: A case report. Arh Hig Rada Toksikol 2008;59:31-5.
10. Mancini G, Carbonara AO, Heremans JF. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965;2:235-54.
11. Metcalf JA, Gallin JI, Nauseef WM, Root RK. Laboratory Manual of Neutrophil Function. Vol. 2. New York: Raven; 1986.
12. Hussain AB. Study of some immunological criterions against some antigen of *Boophilus annulatus* in local rabbits. Al-Anbar J Vet Sci 2011;4:117-24.
13. Wang, T, Long X, Liu Z, Cheng Y. Effect of copper nanoparticles and copper sulphate on oxidation stress, cell apoptosis and immune responses in the intestines of juvenile Epinephelus coioides. Fish Shellfish Immunol 2015;44:674-82.
14. Mohseni M, Pourkazemi M, Bai SC. Effects of dietary inorganic copper on growth performance and immune responses of juvenile beluga, Huso huso. Aquac Nutr 2014;20:547-56.
15. Leeson S. Copper metabolism and dietary needs. Worlds Poult Sci J 2009;65:353-66.
16. El Basuini MF, El-Hais AM, Dawood MA, Abou-Zeid AE, El-Damrawy SZ, Khalafalla MM, *et al.* Effect of different levels of dietary copper nanoparticles and copper sulfate on growth performance, blood biochemical profiles, antioxidant status and immune response of red sea bream (*Pagrus major*). Aquaculture 2016;455:32-40.
17. Vincent M, Hartemann P, Engels-Deutsch M. Antimicrobial applications of copper. Int J Hyg Environ Health 2016;219:585-91.
18. Wang C, Wang MQ, Ye SS, Tao WJ, Du YJ. Effects of copper-loaded chitosan nanoparticles on growth and immunity in broilers. Poult Sci 2011;90:2223-8.
19. Xiao S, Yosef N, Yang J, Wang Y, Zhou L, Zhu C, *et al.* Small-molecule RORγt antagonists inhibit T helper 17 cell transcriptional network by divergent mechanisms. Immunity 2014;40:477-89.
20. Sharma MC, Joshi C, Pathak NN, Kaur H. Copper status and enzyme, hormone, vitamin and immune function in heifers. Res Vet Sci 2005;79:113-23.
21. Zvyagintseva OV, Klimova EM, Lavinska OV, Lenkevich AS. Changing metabolic functions in experimental animals after introduction of the xenobiotic, immunotropic drug and probiotic. Ann Mechnikov Inst 2015;2:125-30.
22. Handy RD. Chronic effects of copper exposure versus endocrine toxicity: Two sides of the same toxicological process? Comp Biochem Physiol A Mol Integr Physiol 2003;135:25-38.
23. Kulseng B, Skjåk-Braek G, Følling I, Espevik T. TNF production from peripheral blood mononuclear cells in diabetic patients after stimulation with alginate and lipopolysaccharide. Scand J Immunol 1996;43:335-40.
24. Reidy T, Rittenberg A, Dwyer M, D'Ortona S, Pier G, Gadjeva M, *et al.* Homotrimeric macrophage migration inhibitory factor (MIF) drives inflammatory responses in the corneal epithelium by promoting caveolin-rich platform assembly in response to infection. J Biol Chem 2013;288:8269-78.
25. Asif HA. Immunological and Physiological Study of Some Manifestations Resulting from Injection of Antigens from *Pseudomonas aerugenosa* Attenuated by *Capparis spinosa* in Mice. Tikrit: College of Science University of Tikrit; 2017.
26. Scott A, Vadalasetty KP, Łukasiewicz M, Jaworski S, Wierzbicki M, Chwalibog A, *et al.* Effect of different levels of copper nanoparticles and copper sulphate on performance, metabolism and blood biochemical profiles in broiler chicken. J Anim Physiol Anim Nutr (Berl) 2018;102:e364-e373.
27. Samanta B, Biswas A, Ghosh PR. Effects of dietary copper supplementation on production performance and plasma biochemical parameters in broiler chickens. Br Poult Sci 2011;52:573-7.
28. Rojas-Sobarzo L, Olivares M, Brito A, Suazo M, Araya M, Pizarro F, *et al.* Copper supplementation at 8 mg neither affects circulating lipids nor liver function in apparently healthy Chilean men. Biol Trace Elem Res 2013;156:1-4.
29. Padrilah SN, Ahmad SA, Yasid NA, Sabullah MK, Daud HM, Khalid A, *et al.* Toxic effects of copper on liver and cholinesterase of *Clarias gariepinus*. Environ Sci Pollut Res Int 2017;24:22510-23.
30. Mitra S, Keswani T, Dey M, Bhattacharya S, Sarkar S, Goswami S, *et al.* Copper-induced immunotoxicity involves cell cycle arrest and cell death in the spleen and thymus. Toxicology 2012;293:78-88.

Source of support: Nil; Conflict of interest: None Declared